The coming of age of biological electron microscopy

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Three-dimensional electron microscopy of macromolecular assemblies by Joachim Frank, *Academic Press*, 1996, *360 pages*, $85.00 hardback (ISBN 0-12-265040-9).

Towards the end of the century, the growth in power and speed of the three major techniques of macromolecular-structure determination shows no sign of slackening. There is not yet an example of an atomic structure of a biological molecule independently determined by all three methods of X-ray crystallography, nuclear magnetic resonance (NMR) and electron microscopy, but there is considerable convergence. For individual, smaller, protein molecules and occasionally complexes, structures are now frequently determined successfully by both X-ray diffraction and NMR, though each method has its realm of dominance. For large macromolecular assemblies such as viruses, a low resolution-structure analysis by three-dimensional electron microscopy is frequently followed by a high resolution X-ray crystal structure determination. In a small number of cases, atomic models of membrane proteins have been derived by electron microscopy alone. With rapid progress being made in all three methods, it cannot be long before the same structure is determined independently by all three. There is however, a unique dearth of books treating the electron microscopy of biological molecules. Joachim Frank's new book *Three-dimensional Electron Microscopy of Macromolecular Assemblies* now puts this right. Frank's book does not attempt the global dominance aimed for by Wuthrich's *NMR of Proteins and Nucleic Acids* nor the finely structured comprehensive coverage of Blundell and Johnson's *Protein Crystallography* but instead introduces the subject from the author's rich personal perspective. Frank's background and experience are in the theory of image formation in electron microscopy, the development of mathematical methods for computer-based image processing in two and three dimensions and their application at increasingly higher resolution to the structure determination of macromolecular assemblies, especially the ribosome. A beautiful three-dimensional multicoloured representation of the *E. coli* ribosome in the act of translating an RNA message into a growing polypeptide is shown on the cover, derived using the methods described in the following 300 pages.

Between the covers, the text explains clearly all the jargon and keywords necessary for entry to the brotherhood of biological electron microscopy. It contains a modicum of mathematics, in just about the right dose for biologists. Frank has a tendency to blur over critical problems by wordy definitions and philosophical musing, but this adds to the unique and attractive character of the book.

On the technical side, the presentation is slanted towards image-analysis methods which involve classification, principal-component analysis and multivariate statistics. There is a strong emphasis on mathematical power as the dominant approach to overcoming the physical imperfections of the images. As in a Simenon detective story, reality emerges from the clues that are disentangled through philosophically oriented mathematical exploration.

The discussion of the improvements in resolution attained during the development of the field, from the negatively stained specimens of the 1960s through to the unstained, ice-embedded procedures introduced by Dubochet and his colleagues in the 1980s, is well covered. There are excellent in-depth explanations of the various resolution criteria (differential phase residual [DPR], Fourier ring correlation [FRC], ratio of moduli of vectorial sum to sum of moduli of contributing structure factors [Q factor]) adopted by different factions, as well as rehearsals of earlier, illuminating arguments and controversies. Frank himself seems unduly pessimistic about the long-term possibilities for atomic resolution single-particle structure determinations of macromolecular assemblies, or 'crystallography without crystals' as he calls it. He does not mention the possibility of restoring the power of high resolution Fourier components, which are greatly reduced at the outer resolution limit of any analysis by simply 'sharpening' the data. Instead, he emphasizes the very small power content at the outer resolution boundary and seems to suggest that such measurements, which are statistically absolutely reliable, are somehow less valuable because they are weak in the image: there is no mention that a restoration of the original power can be done by simple multiplication.

The book is nevertheless a wonderful, coherent and satisfying introduction to the field. Its publication adds to the underlying framework of structures supporting the field which now boasts its own Gordon Conference series *Three-dimensional EM of Macromolecules* held every two years (the next meeting is in 1997); its own e-mail mailing list 3dem@mcrcr6.med.nyu.edu (to subscribe, send 'subscribe 3dem' to mailserv@mcrcr.med.nyu.edu), a 3dem web page http://rcr-www.med.nyu.edu/3dem; and a recent special issue of *Journal of Structural Biology* (**116**, 1–249 [1996])

with 33 articles describing all of the currently available software in the field. Amongst these, there is an article on the SPIDER/WEB system that Frank's group has pioneered, which is used for most of the illustrations in the book. This book should be a compulsory addition to the shelves of all those structural biologists who aim for a wider understanding of the methods of macromolecular structure determination.